PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Schillinger *et al.*	Group Art Unit: 1661
Serial No.: 10/052,771	Examiner: Para, A.
Filed: January 23, 2002	Atty. Dkt. No.: ASGR:002USD1
For: PLANTS HAVING RESISTANCE TO MULTIPLE HERBICIDES AND ITS USE	

CERTIFICATE OF MAILING
37 C.F.R. §1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: ~~Assistant~~ Commissioner for Patents ~~Washington, DC 20231~~, on the date below:

10/13/06
Date

Robert E. Hanson

DECLARATION OF DR. JOSEPH R. BYRUM UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, JOSEPH R. BYRUM HEREBY DECLARE AS FOLLOWS:

1. I am a U.S. citizen and currently reside at 913 31st Street, West Des Moines, Iowa.

2. I have been employed by Asgrow Seed Company, Inc., and Monsanto Company, the parent company of Asgrow Seed Company, Inc., since 1995; currently with the title of Director, Soybean Trait Integration.

3. I hold a Ph.D. in Plant Breeding and Genetics from Iowa State University and a B.S. in

Crop and Soil Sciences from Michigan State University. I have been conducting research in the area of plant biochemistry, molecular biology, and genetics since 1989. My duties have included the creation of transgenic crop varieties since 1993.

4. I am an inventor of the above-captioned patent application and am familiar with the contents of the patent application.

5. I understand that the Patent and Trademark Office Examiner in charge of assessing the patentability of the referenced patent application has rejected the claims as being obvious. In particular, it is my understanding that it has been asserted that it would have been obvious to engineer resistance to the herbicides glyphosate and glufosinate in a single soybean plant. As noted by the Examiner, I previously submitted a declaration in this case related to this issue, on May 25, 2004.

6. I am therefore providing a second Declaration to submit additional information demonstrating that it would not have been obvious to those of ordinary skill in the field of agriculture and biotechnology at the time the application was filed to produce a single soybean plant exhibiting resistance to glyphosate and glufosinate.

7. As indicated previously, at the time of the invention a soybean variety had not been developed having more than one herbicide resistance trait. Researchers in the area, meanwhile, were studying the reported effect on soybean yield of a single herbicide tolerance (*i.e.* glyphosate tolerance) transgene. Thus any effect on soybean yield of the presence of more than one herbicide tolerance transgene remained to be studied. Any prior assertions that glyphosate and glufosinate herbicide resistance transgenes could be successfully expressed in a single variety while maintaining a commercially acceptable soybean grain yield would

therefore be speculation. Specifically, at or around the filing date of the present application, researchers in the area would have been aware of published reports of "yield lag" and "yield drag" which were being intensively studied in Roundup Ready™ ("RR") glyphosate resistant soybean cultivars. "Yield lag" has been defined as a reduction in yield due to the background genetics of a transgenic soybean cultivar or line, while "yield drag" was thought by some to be due to an unidentified effect of the transformation process or an effect of the transgene itself.

8. While the accuracy of the reports was subsequently called into question (*e.g.* Breitenbach & Hoverstad, *Crop News* 4:162-163 (1998)), discussions in the art as of the filing date asserted that, on average, the seed yield of RR soybean was apparently lower than that of non-transgenic sister lines, although the precise cause was unclear. Research carried out in 1995-1997 (*e.g.* as cited in Raymer *et al.,* 2003 (*Crop Sci.* 43:1584-1589; attached as EXHIBIT 1); Anon., 1996 (Missouri Agricultural Experiment Station, Hundley-Whaley Farm Newsletter attached as EXHIBIT 2)); and as described in Marking, 1999 (*Soybean Digest* March 1999, attached as EXHIBIT 3) continued to note this apparent effect. For instance, Raymer *et al.* stated (paragraph bridging pp. 1585-1586 in EXHIBIT 1):

> A second issue that quickly arose following the introduction of RR cultivars was related to concerns that yields of RR cultivars were not competitive with yields of conventional cultivars. Raymer (1997) reported on the performance of RR cultivars entered in university SVT ["soybean variety test"] conducted in nine southern states during 1995 and 1996. Although yields of some RR cultivars in some trials were often above the mean yield of the trial, seed yields of the population of RR cultivars as a whole averaged 2.9 units below the mean (Fig. 2) . In comparison, seed yields of conventional southern regional check cultivars averaged 1.0 unit above the trial mean and 3.9 units higher than the average of all RR entries.

A November 1996 newsletter of the University of Missouri Agricultural Experiment Station (attached as EXHIBIT 2) stated:

> Do Roundup Ready Varieties Have the Genetic Ability to Yield as Well as the Top Non-transformed Varieties? This year there was a statistically significant difference in soybean seed yield between the transformed and non-transformed variety in the test. Untreated Pioneer 9362 yielded 52.8 bushels/acre. Untreated Pioneer 9363RR yielded 46.6 bushels/acre. Since the LSD was 4.6 bushels/acre, the data indicates a statistically higher yield for the non-transformed soybean variety...
>
> In summary, the data generated by this study raise the question of whether there is a "yield drag" with Roundup Ready™ soybeans. In this work, there was some indication that this was the case, but one year's data should not be used to make long term decisions. It does raise a flag that warrants further work in the selection of transformed verses non-transformed crop varieties. It should also provide producers a reason to be cautious about large scale adoption of this weed control system without some personal experience with it in their own operation.

Marking (1999; EXHIBIT 3, page 1), describing general knowledge from 1996 onward, stated:

> * When averaged across all tests, RR varieties were 4% lower in yield than conventional varieties. That would be a 2-bu/acre difference on a 50-bu/acre yield.
>
> * When averaged across all locations, the top five RR varieties yielded 5% less than the top five conventional varieties in 200 comparisons.
>
> Harry Minor, a University of Missouri extension agronomist, made similar comparisons in his state and came up with about the same yield lag - 1.6 bu/acre. In some comparisons he made of Group V beans from Arkansas and Mississippi, there was about a 5% yield lag with RR varieties.
>
> Oplinger adds that another University of Wisconsin study used so-called sister lines - very similar genetics with the RR gene in one of the matched pairs. The yield difference in favor of the conventional varieties was 4-6%.
>
> This yield lag factor for RR soybeans has been mentioned by many scientists and top-end growers who made side-by-side comparisons since 1996, when these transgenic varieties were first commercialized. And it was mentioned in each of Soybean Digest's Special Reports on Herbicide-Tolerant Crops since 1996.

Thus the attached exhibits show that one of ordinary skill in agriculture and biotechnology at the very least would have been aware of numerous reports alleging that a glyphosate tolerance transgene reduces yield and would understand that addition of a herbicide tolerance transgene to soybean while maintaining an acceptable yield was <u>by no means a straightforward process</u>. Further, addition of yet another transgene, conferring glufosinate tolerance, would be expected to exacerbate this issue.

9. Based on the foregoing, a person of ordinary skill in agriculture and biotechnology would have been without any reasonable expectation at the time the application was filed that introducing glyphosate <u>and</u> glufosinate resistance transgenes into a single soybean plant would yield a plant that is resistant to both herbicides while maintaining a commercially significant yield.

10. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

10/02/2006
Date

Joseph R. Byrum
Joseph R. Byrum

Challenges in Comparing Transgenic and Nontransgenic Soybean Cultivars

Paul L. Raymer* and Timothy L. Grey

ABSTRACT

Genetically modified soybean [*Glycine max* (L.) Merr.] cultivars containing herbicide resistance have become the predominant cultivars marketed in the USA. This technology dramatically changed weed management strategies in the USA and created controversy and new challenges within various segments of the industry. This review seeks to summarize many of the issues that arose within the soybean industry surrounding the introduction of the world's first major transgenic crop. Hopefully, the experiences gained during this transition period will benefit future efforts to commercialize transgenic crops. Initially, the new transgenics made the already difficult, yet critically important, task of cultivar selection even more arduous and confusing. Lack of university data, high cultivar turnover rates, and the added burden of factoring in technology fees, yield lag, and weed control costs into the decision of which cultivar to plant were frustrating to most producers. Industry's early insistence on separate cultivar performance trials for Roundup Ready (RR) cultivars taxed limited testing resources and increased the size of the already massive data sets generated. Most often, the end result did not provide a direct comparison with popular conventional cultivars. As this new technology has become fully accepted and assimilated into our industry, comparisons with conventional cultivars are less important since most farmers are selecting only among RR cultivars. The current literature indicates that soybean variety test (SVT) programs with limited resources can effectively compare soybean cultivar performance using combined trials treated only with conventional herbicides with little risk of yield loss or herbicide × cultivar interactions. However, the use of a systems approach or separate RR trials to evaluate the performance of soybean cultivars is now commonplace in the USA due to the sheer dominance of RR cultivars. Many larger SVT programs will soon consider discontinuing separate trials for conventional cultivars as their numbers and importance diminish.

THE PHRASE *genetically modified organism*, or its more commonly known acronym GMO, is now a household word. Ten years ago, few people in the USA knew the meaning of the term; now most Americans have at least some understanding of the term and the many consumer and environmentally related issues that surround it. GMO crops, also known as transgenic crops, are now commonplace in American agriculture. These terms broadly refer to crop cultivars that were developed using recombinant DNA techniques and that contain genetic material from another organism. The use of transgenic crops that express traits considered advantageous for pest management has risen dramatically since their commercial introduction in the mid-1990s. In soybean, the dominant transgenic technology currently in use is the RR system. Roundup Ready soybean cultivars contain a bacterial transgene, commonly known as CP4, that confers resistance to glyphosate (N-phosphonomethyl glycine), the active ingredient in Roundup. Glyphosate-resistant soybean cultivars were first commercially introduced in the USA in 1996 (Fig. 1). More than 400 thousand hectares of RR soybean were planted in the USA the first year it was commercially available (Fernandez-Cornejo and McBride, 2000). In 2001, the USDA estimated 68% of the 30 million hectares of soybean in the USA were planted to RR cultivars (National Agricultural Statistics Service, 2001).

The rapid adoption rate of this system infers soybean producers believe it offers advantages over the conventional weed control systems they were using. Results of a farmer survey conducted by the USDA regarding adoption of genetically engineered crops were summarized by Fernandez-Cornejo and McBride (2000). Sixty-five percent of growers surveyed indicated they used the RR soybean system because of the potential for increased yields through improved pest control. Another 20% indicated the primary reason was decreased pesticide cost. Certainly this system does offer the potential for enhanced weed control. Roundup is a broad-spectrum herbicide that controls most grasses and many problematic broadleaf weeds common in soybean production (Delannay et al., 1995). It is also possible for growers using this system to reduce herbicide costs (Fernandez-Cornejo and McBride, 2000) and improve flexibility in timing of herbicide application (Vangessel et al., 2000) since many weed species can be controlled much later in the growing season than was previously possible using conventional herbicides. Soybean lines with the RR system have good crop tolerance to glyphosate and therefore, afford less opportunity for herbicide-related crop injury. The combination of the above factors makes this system of weed control easier for the farmer to manage. Ease of management is most likely the actual reason this technology was adopted so quickly.

Separate Trials for Roundup Ready Cultivars

Shortly after the introduction of the RR system, several industry-related issues arose. The first issue was precipitated by Monsanto's early insistence that only Roundup should be used on RR cultivars. Monsanto justified this position by contending conventional herbicides injured soybean and reduced seed yield. Therefore, the full benefit of the RR system could not be realized unless only Roundup was used on RR cultivars. As a result of this position, Monsanto-owned seed companies refused to enter RR cultivars in conventional trials or in any trials that herbicides other than Roundup would be used. Many agronomists within the soybean industry viewed this position as a corporate attempt to

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Abbreviations: GMO, genetically modified organism; RR, Roundup Ready; SVT, soybean variety test.

conceal deficiencies in cultivars containing this new technology.

This issue created a serious dilemma for SVT programs throughout the country. If a SVT program failed to modify its structure to specifically accommodate RR cultivars, most seed companies would not enter their cultivars and limited RR cultivar performance information would be obtained. This compromised the very purpose of university-operated testing programs, since their primary mission is to provide unbiased cultivar performance data to aid growers in selecting cultivars.

On the other hand, if an SVT program established separate RR cultivar trials, they were in essence duplicating their existing programs and taxing limited program resources. This is what most university testing programs eventually did, although with some delay. Even though RR cultivar performance data were being generated and disseminated, its usefulness to growers was greatly diminished. The establishment of separate RR trials precluded valid direct comparisons between conventional and RR cultivars. At the time, a direct comparison between RR and conventional cultivars was the single biggest piece of information that growers needed to determine how this new technology compared with the technology that they were currently using.

A third option first used by testing programs in Missouri and later in Georgia and a few other states was to include conventional cultivars as checks for comparison in Roundup-treated trials (Minor, 1998). This approach represented a compromise between the first two options discussed previously and required that conventional checks be protected when Roundup was applied. Initially this appeared to be a logical way around the problem. Only Roundup was applied to RR cultivars, and yet comparisons with conventional cultivars were possible. However, this protocol proved difficult to manage and often produced unreliable data. Conventional check plots required spot treatment with conventional herbicides or hand weeding. Untimely weed control often resulted in competition that biased results. Furthermore, the end result of even the most well-managed trial was a limited set of comparisons between the RR cultivars and a few conventional checks.

The University of Illinois program (Table 1) illustrates the impact of the introduction of RR cultivars on the structure and size of programs (R. Esgar, 2001, personal communication). In 1996, at the time of the introduction of RR technology, the University of Illinois offered soybean cultivar tests at nine locations. In 1997, even though RR cultivars were widely available to producers, the University of Illinois still offered only conventional trials at all nine locations. Of the 607 cultivars tested by the University of Illinois in 1997, only three (0.5%) were RR. In 1998, separate RR trials were established at five of the nine locations, increasing the total number of trials offered to 14. Seed companies responded quickly to these additional trials. The number of RR cultivars tested increased from three the previous year to 328 and the total number of cultivars tested increased from 607 the previous year to 807. The University of Illinois modified its testing structure in 1999 to offer both RR and conventional trials at each of the nine locations. The number of RR cultivars tested increased to 451 and surpassed the number of conventional cultivars tested. In 2000, the number of test locations increased to 12 with both RR and conventional trials offered at each location. This brought the total number of trials offered to 24, an increase of 266% since the introduction of the RR cultivars. In 2001, 75.9% of the 681 soybean cultivars tested by the University of Illinois program were RR.

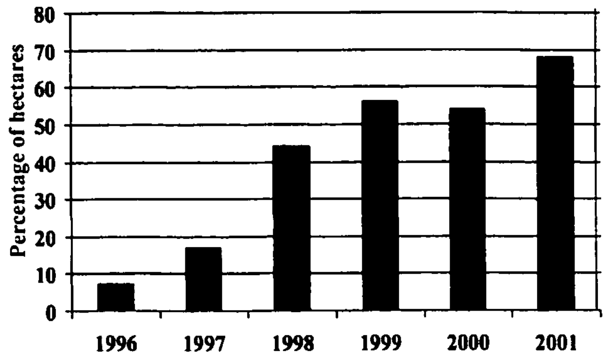

Fig. 1. Adoption of Roundup Ready soybean in the USA, 1996 to 2001. Source: National Agricultural Statistics Service, 2001.

Yield Depression of Roundup Ready Cultivars

A second issue that quickly arose following the introduction of RR cultivars was related to concerns that yields of RR cultivars were not competitive with yields of conventional cultivars. Raymer (1997) reported on the performance of RR cultivars entered in university SVT conducted in nine southern states during 1995 and 1996. Although yields of some RR cultivars in some

Table 1. Number of trial locations and number of conventional and Roundup Ready cultivars entered in the University of Illinois Soybean Trials, 1996 to 2001.

	1996	1997	1998	1999	2000	2001
			No. trial locations			
Conventional	9	9	9	9	12	12
Roundup Ready	0	0	5	9	12	12
Total trials	9	9	14	18	24	24
			No. cultivars entered			
Conventional	633	604	479	323	335	164
Roundup Ready	2	3	328	451	447	517
Total entries	635	607	807	774	772	681
% Roundup Ready cultivars	0.3	0.5	40.6	58.3	57.9	75.9

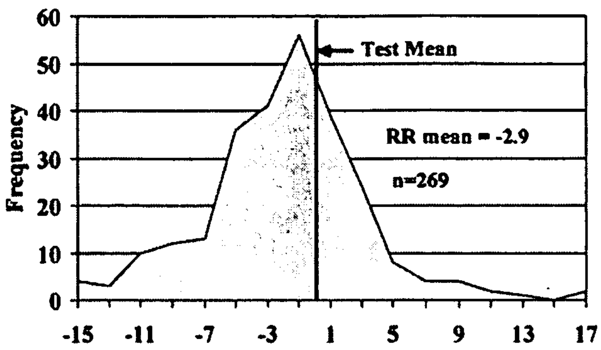

Fig. 2. Frequency distribution of 269 individual comparisons of the seed yield of Roundup Ready (RR) soybean cultivars relative to the test mean. Data are from soybean variety test programs conducted by nine southern states in 1995 and 1996. Each unit on the *x*-axis represents a difference of 67.19 kg ha^{-1} (one bushel per acre).

trials were often above the mean yield of the trial, seed yields of the population of RR cultivars as a whole averaged 2.9 units below the mean (Fig. 2). In comparison, seed yields of conventional southern regional check cultivars averaged 1.0 unit above the trial mean and 3.9 units higher than the average of all RR entries. Minor (1998) reported similar findings from southern SVTs.

In 1999, the 1995 and 1996 data discussed above were compared with performance data from university trials conducted in 1998 (Raymer and Minor, 1999). Analysis of the 1998 data indicated that average performance of RR cultivars had improved. Seed yields of RR cultivars in 1998 were only 0.5 units below the trial mean as compared with 2.9 units below the trial mean in the 1995-1996 data set. However, the average yield of RR cultivars was still 1.5 units below that of the conventional check cultivars. Examination of the 1998 data by maturity group indicated yield parity for RR Maturity Group IV cultivars had been reached in that they performed as well as conventional cultivars. There was still evidence of a small yield lag persisting in Maturity Groups V through VIII. It was also noted that disease and nematode resistance were often compromised in RR cultivars, as many RR cultivars lacked the level of disease resistance or nematode resistance of their conventional counterparts. On the basis of the trend indicated by these data, the authors concluded that with continued breeding efforts, RR cultivars would soon become fully competitive with conventional cultivars.

Oplinger et al. (1998) reported significantly lower yields of RR cultivars when the top five RR cultivars were compared with the top five conventional cultivars entered in the 1998 SVT programs of nine northern states. Christmas of Purdue University made similar comparisons using 1999 data from eight northern state SVT programs and reported a lower but still significant yield penalty for RR cultivars when compared with non-RR cultivars (Nielsen, 2000).

Technology Lag or Technology Drag

Once it became apparent that lower yields were often associated with RR cultivars, many in the industry began to propose possible explanations. Most explanations fell into two main areas and the issue became popularly referred to as *technology lag* vs. *technology drag*. The term *technology lag* implies a temporary or transient problem associated with the introduction of a new technology, in this case resistance to glyphosate. Eventually the industry would be expected to overcome the problem. The potential for a yield lag would be expected to increase when (i) the trait of importance is from a single genetic source, and (ii) it is rapidly introgressed into a broad range of highly advanced germplasm. In the case of glyphosate resistance, the original registered transformation event was performed on 'Asgrow A5403', a well-adapted and high yielding cultivar. This cultivar became the only source of glyphosate resistance for all breeding programs.

Some speculated that at least a portion of the problem could be attributed to old genetics. Many of the initial RR cultivars released were developed using a backcross breeding approach to introgress the glyphosate resistance gene into proven top performing cultivars. A backcross program is a 3- to 5-yr process for even the fastest breeding program. The original cultivar used as the recurrent parent in many cases was 10 yr old or more by the time the RR version became commercially available. It is possible that the newly released RR cultivar with old genetics had now been surpassed in performance by the most recently developed conventional cultivars. Still others insisted the evaluation period for the first RR cultivars was abbreviated and yield too often compromised by breeders and seed companies in their haste to commercialize the first wave of RR cultivars.

The term *technology drag* implies an inherent yield reduction associated with the technology itself. The concept of yield drag is certainly of more concern than yield lag because drag implies a permanent and perhaps unavoidable problem directly related to the technology. Possible explanations for yield suppression that fall into the technology drag category are: (i) the presence of the gene (CP4 conferring glyphosate resistance) reduces fitness of the plant, (ii) normal genetic or physiological processes were disrupted by the transformation process, and (iii) the application of glyphosate causes yield suppression.

Delannay et al. (1995) reported results from over 60 field trials and conclusively stated that glyphosate application did not affect seed yield of RR soybean lines and that the RR soybean lines were not different in seed yield than their nontransgenic parental lines. These findings provided strong evidence against the possibility of a technology drag. Elmore et al. (2001a) also showed that no yield effect was associated with application of glyphosate on RR cultivars. However, in a companion paper, Elmore et al. (2001b) reported that RR sister lines yielded 5% less than the non-RR sisters. They also included three high-yield, non-RR cultivars, and five other RR cultivars in the comparison and reported that the high-yield, non-RR cultivars yielded 5% more than the non-RR sisters and 10% more than the RR sisters. On the basis of these findings they concluded that a 5% yield suppression was related to the gene or its insertion

process and another 5% suppression was due to cultivar genetic differential. On the basis of these recent reports, it appears that the existence of a technology drag associated with the RR system will continue to be debated.

Cultivar Selection and Transgenics

The primary objective of university-operated SVT programs is to provide accurate and unbiased performance information for comparing the cultivars that are available to producers. The SVT-generated data provides a multiyear database that hopefully will aid the producer in selecting the best-suited cultivar and improving profits. Initially, the new transgenics made the already difficult yet critically important task of cultivar selection even more arduous and confusing. This process suddenly became more difficult for both the extension specialist who attempted to present data to facilitate the decision-making process as well as for the producer who was making the decision.

First, SVTs generated limited performance data on RR cultivars in 1996, 1997, and 1998 because few RR cultivars were entered. As a result, growers often did not have the information they needed to make educated decisions regarding cultivar selection. Once RR cultivars began to be entered in SVT programs, many programs noted that cultivar turnover rates increased dramatically. In the Georgia SVT, the percentage of cultivars entered for two consecutive years decreased from 70% in 1994 to 46% in 1998 (Day, 2001, personal communication). Companies were rushing to get their latest, best-yielding RR cultivars out to growers, and often RR cultivars were only marketed for 1 yr before being replaced. High turnover rates made it difficult to amass multiyear data that is often necessary to make accurate decisions on cultivar performance (Bowman, 1998).

The introduction of herbicide-resistant technology greatly complicated the actual process of cultivar selection. With the advent of herbicide-tolerant cultivars, growers now had to factor into the cultivar decision-making process such things as technology fees, premiums on seed costs, and potential differences in weed control costs. Concerns that growers might give up yield potential or needed pest resistance if they selected a RR cultivar were also complicating issues.

With the introduction of herbicide-tolerant cultivars, seed-associated costs and weed-control costs were no longer separate issues, they were now interrelated and must be considered together. Even when total seed-associated costs and weed-control costs appeared comparable between RR and conventional systems, soybean farmers often gave up yield potential by selecting less competitive RR cultivars, and as a result actually reduced their potential for profit. Growers desiring to use the RR system must select the highest-yielding and best-adapted RR cultivars available to maintain maximum profitability.

The cultivar selection decision before the introduction of RR cultivars was relatively straightforward. Among all available cultivars, producers selected cultivars within maturity groups most adapted to their area, and then within that set of cultivars they selected those cultivars with the pest resistance(s) needed. Within that subset of cultivars they selected the highest yielding cultivar(s) for their farm. The decision of what weed control program to use was an unrelated and separate process.

Now with the large-scale adoption of RR cultivars and the availability of yield-competitive RR cultivars comes an opportunity to alter the decision-making process and to simplify things greatly by placing the decision of what weed control system to use at a level above the cultivar selection decision (Fig. 3). Using this approach, producers first select the weed control program for their farm. That decision should be field-specific, based on the weed spectrum present, and consider the associated costs and the level of management the producer desires. Once the weed control system is determined, only cultivars that are appropriate for that system are considered for planting. Producers should strive to select the most cost-effective weed control program and then select the highest-yielding cultivars that are available for use in

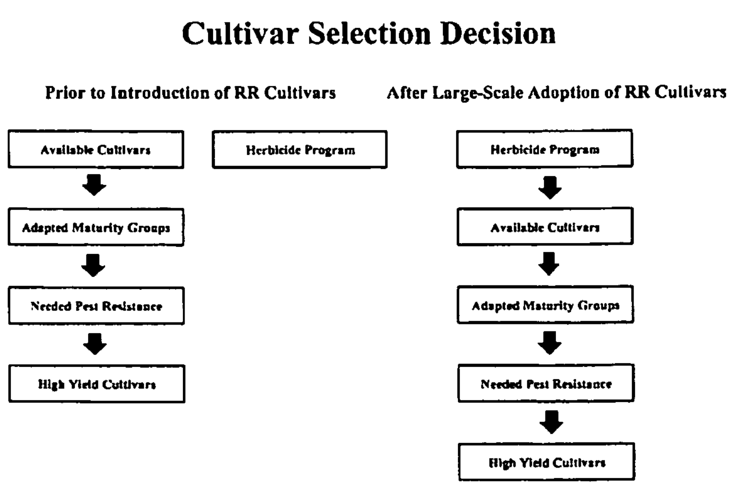

Fig. 3. Schematic representation of the cultivar selection process before and after large scale adoption of Roundup Ready (RR) soybean.

that program. This approach negates the need for direct comparisons of RR cultivars to conventional cultivars or certainly makes those comparisons less important than they were when RR cultivars were first introduced.

Injury of Roundup Ready Soybeans by Conventional Herbicides

Probably the only valid justification for separate cultivar trials or a system approach for the evaluation of RR cultivars is the contention that conventional herbicides reduce yields of conventional as well as RR soybean cultivars. Starting in 1999, journal articles addressing this issue began to appear in the literature. Comparisons among glyphosate only, residual herbicides only, the combination of these two systems, and in some cases, comparisons with hand-weeded controls have been reported (Gonzini et al., 1999; Krausz and Young, 2001; Krausz et al., 2001). Glyphosate alone (Gonzini et al., 1999; Akin and Shaw, 2001; Culpepper et al., 2001; Krausz and Young, 2001; Krausz et al., 2001; Wiesbrook et al., 2001) and in combinations with alachlor (Methoxymethyl-2′,6′-diethylanilide chloroacetate) (Gonzini et al., 1999; Krausz et al., 2001), chlorimuron {2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl) amino] carbonyl] amino] sulfonyl] benzoic acid ethyl ester} (Akin and Shaw, 2001; Gonzini et al., 1999), clomazone [2-((2-chlorophenyl) methyl)-4,4-dimethyl-3-isoxazolidinone] (Wiesbrook et al., 2001), cloransulam-methyl {N-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro (1,2,4) triazolo-[1,5-c]pyrimidine-2-sulfonamide} (Gonzini et al., 1999; Krausz and Young, 2001), imazaquin [2-(5-isopropyl-5-methyl-4-oxo-2-imidazolin-2-yl)] (Akin and Shaw, 2001; Krausz and Young, 2001), imazethapyr [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-5-ethyl-3-pyridinecarboxylic acid] (Gonzini et al., 1999; Krausz and Young, 2001; Wiesbrook et al., 2001), metolachor [2-Chloro-6'-ethyl-N-(2-methoxy-1-methylethyl)-o-acetoluidide] (Akin and Shaw, 2001), metribuzin [4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] (Gonzini et al., 1999), pendimethalin [N-(Ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] (Gonzini et al., 1999; Krausz and Young, 2001; Wiesbrook et al., 2001), and sulfentrazone (3-Phenoxybenzyl 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropanecarboxylatė) (Gonzini et al., 1999; Akin and Shaw, 2001) have also been investigated.

Yield reductions associated with the use of residual herbicides on RR soybean cultivars have been most often reported when using imidiazolinone chemistry. Gonzini et al. (1999) reported that average soybean yield for imazethapyr alone (preemergent) treatments was 79% of the hand-weeded control. Wiesbrook et al. (2001) reported that yield of imazethapyr (postemergence) in combination with pendimethalin treatment was 83% of the glyphosate treatment. Akin and Shaw (2001) noted imazaquin with metolachlor averaged 82% of the yield of the glyphosate treatment. Other reports indicated little or no yield reduction associated with the use of imidazolinone chemistry (Krausz and Young, 2001; Krausz et al., 2001).

Although some reports, as referenced above, support claims of yield reduction due to injury by conventional herbicides, Wiesbrook et al. (2001) pointed out that yields may be reduced as a result of increased weed competition in glyphosate-tolerant soybean systems that utilize only residual herbicides for weed control. To conclusively determine if yield losses are due to injury by conventional herbicides rather than differences in weed competition between the two systems, hand-weeded residual herbicide treatments must be compared directly to hand-weeded glyphosate treatments. Such comparisons have not been reported.

Contrary to the above concerns of injury from conventional herbicides, a number of published reports suggest that the use of conventional herbicides in combination with glyphosate may improve weed control and yield. Some weed species, specifically morning-glory (*Ipomoea* spp.), have some level of tolerance to glyphosate (Culpepper et al., 2001; Wiesbrook et al., 2001; Grey and Raymer, 2002). Weed escapes can occur in a glyphosate-only system due to the lack of residual activity, and these weedy escapes can reduce yield (Swanton et al., 2000). Thus, efforts to control some weed species have emphasized the use of soil residual herbicides in combination with glyphosate. Other research indicates that use of preplant soil-applied herbicides can also be advantageous in the RR system by providing a wider glyphosate application window (Vangessel et al., 2000).

Regardless of whether the use of conventional herbicides on RR cultivars causes injury and reduced yield or actually complements glyphosate use; this issue has largely disappeared from the industry. Monsanto has now modified its position on this matter as evidenced by the facts that it has labeled a number of Roundup-tank mixes, and seed companies are now very willing to enter RR cultivars in combined SVT trials where only conventional herbicides are used.

Current Status of Roundup Ready Cultivars in Soybean Variety Tests

Of 27 states surveyed, 20 now offer separate RR trials and seven offer only combined trials where RR and conventional cultivars are tested side-by-side using only conventional herbicides. With the exception of Arkansas, most of the SVT programs that offer only combined trials are relatively small and have difficulty justifying separate trials.

In 1997, only conventional trials were offered at Illinois (Table 1) and 607 different cultivars were tested in their program. Total number of cultivars tested peaked at 807 in 1998, the first year RR-only trials were offered. Total cultivars tested declined from 1999 to 2001 and are now approaching levels tested before the establishment of separate RR trials. The number of conventional entries declined as the proportion of RR cultivars entered increased from 1997 to 2001. In 2001, 76% of the cultivars entered in the Illinois SVT were RR cultivars. Although separate trials for RR soybean cultivars may or may not be justified based on the potential for herbicide injury as discussed above, separate

trials may be well justified by the sheer dominance of RR cultivars entered in SVTs.

Concluding Comments

The transgenic nature of glyphosate resistance is irrelevant to all the issues discussed above. Most of these issues were not created because of the transgenic nature of the herbicide resistance trait, but rather were the direct result of the unprecedented marketing approach and corporate policies surrounding the commercialization of this technology. Fortunately, the majority of these issues have proven to be transient and inherently associated with the transition from the use of conventional cultivars to the use of herbicide-resistant systems. Hopefully, the experiences gained during the introduction of RR soybean cultivars will benefit future efforts to commercialize other transgenic traits.

Research findings in the weed science literature indicate that it is possible for SVT programs to effectively determine soybean cultivar performance in combined trials with little risk of yield loss or herbicide × cultivar interactions due to the use of conventional herbicides. The current dominance of RR cultivars in U.S. production minimizes the importance of early arguments against the systems approach (separate RR trials) to cultivar evaluation. Direct comparisons with conventional cultivars are now seldom needed since most soybean farmers are selecting only among RR cultivars. The use of a systems approach by SVT programs is now commonplace in the USA. This approach does provide an effective means of generating data for direct comparisons among RR cultivars. As the number and importance of available conventional soybean cultivars diminishes in the U.S. soybean industry, many larger SVT programs will now consider if they can continue to justify separate trials for conventional cultivars.

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FOR THE WEEK OF NOVEMBER 3, 1996

RR BEAN PERFORMANCE: The visual physical response of transformed, Roundup Ready soybeans have been investigated at the Hundley-Whaley farm at Albany for the past five years in cooperation with Monsanto Chemical Company. The data generated from those studies clearly indicate that plants from the resistant lines are not visibly injured by applications of Roundup herbicide. The data also indicates that a total weed management strategy can be developed using only the herbicide Roundup.

Soybean producers are showing a great deal of interest in this concept for several reasons. A major one is the lack of apparent crop damage caused by the Roundup Ready (RR) weed control program. Also, the costs of soybean weed control programs with RR beans have the potential to be markedly reduced in comparison to competitive programs.

Many seed companies, in cooperation with Monsanto, will be introducing Roundup Ready varieties to the farm market in 1997. Since soybean producers need non-biased information to be able to intelligently determine whether to pursue this production strategy, we asked the Missouri Soybean Merchandising Council to fund a research project that would have the objective of answering four questions. They agreed to do so and the study was conducted. It was done under weed-free conditions to prevent weed competition having an effect upon crop response. The results of the study are in. The questions, along with the answers, are as follows.

DOES ROUNDUP APPLICATION FREQUENCY INFLUENCE RR SOYBEAN CROP YIELD?

In this test, Roundup application frequency had no affect on transformed soybean crop yield. We applied as many as three applications of Roundup to the RR beans in this study and found no adverse effect upon soybean height, number of nodes or crop yield.

DOES RATE OF ROUNDUP USED INFLUENCE TRANSFORMED SOYBEAN CROP YIELD?

The rate of Roundup used in this study had no adverse influence on RR soybean crop yields. We made mid-post applications of 0, 2, 3 and 4 pints per acre of Roundup and there were no statistical yield differences. Actual yields varied from 46 to 49 bushels per acre for these treatments.

IS RR SOYBEAN YIELD INFLUENCED BY ROUNDUP APPLICATIONS MADE AT VARIOUS STAGES OF SOYBEAN GROWTH?

Not in this study. One and one-half pints of Roundup was applied early post, mid-post, late-post and very late-post without differences in soybean physical appearance or yield differences. I concluded that neither RR bean yield or physical appearance was adversely influenced by Roundup applications made at various stages of soybean growth.

DO ROUNDUP READ VARIETIES HAVE THE GENETIC ABILITY TO YIELD AS WELL AS THE TOP NON-TRANSFORMED VARIETIES?

This year there was a statistically significant difference in soybean seed yield between the transformed and non-transformed variety in the test. Untreated Pioneer 9362 yielded 52.8 bushels per acre. Untreated Pioneer 9363RR yielded 46.6 bushels per acre. Since the LSD was 4.6 bushels per acre, the data indicates a statistically higher yield for the non-transformed soybean variety.

METHODS: The study consisted of the twelve treatments. Weed control was not an issue in the study. Broadstrike + Dual was pre-emergence applied to all plots in an attempt to get weed control. The plots were also hand weeded for escape weeds during the growing season. The study was designed as a randomized compete block with 6 replications. The study was planted in mid-June.

SUMMARY: The crop yield data generated by this experiment raise the question of whether there is a "yield drag" with Roundup ReadyTM soybeans. In this work, there was some indication that this was the case, but one year's data should never be used to make long term decisions. It does raise a flag that warrants further work in the selection of crop varieties, especially transformed verses non-transformed. It should also provide producers a reason to be cautious about large scale adoption of this weed control system unless they have some personal experience with it in their own operation.

Roundup Ready Yields Lag

Mar 15, 1999 12:00 PM
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Roundup Ready (RR) soybeans have turned in some whopper yield performances, like topping 90 bu/acre in one Ohio State University variety performance test in '98.

But extensive research data now strongly suggest there is, on average, a small yield lag with this new technology.

Data from more than 3,000 side-by-side comparisons from 40 university performance tests conducted across eight states in 1998 were summarized by Ed Oplinger, University of Wisconsin extension agronomist.

Here are the results:

* Average yields of RR varieties ranged from 14% less to 13% more than conventional varieties.

* When averaged across all tests, RR varieties were 4% lower in yield than conventional varieties. That would be a 2-bu/acre difference on a 50-bu/acre yield.

* When averaged across all locations, the top five RR varieties yielded 5% less than the top five conventional varieties in 200 comparisons.

Harry Minor, a University of Missouri extension agronomist, made similar comparisons in his state and came up with about the same yield lag - 1.6 bu/acre. In some comparisons he made of Group V beans from Arkansas and Mississippi, there was about a 5% yield lag with RR varieties.

Oplinger adds that another University of Wisconsin study used so-called sister lines - very similar genetics with the RR gene in one of the matched pairs. The yield difference in favor of the conventional varieties was 4-6%.

This yield lag factor for RR soybeans has been mentioned by many scientists and top-end growers who made side-by-side comparisons since 1996, when these transgenic varieties were first commercialized. And it was mentioned in each of Soybean Digest's Special Reports on Herbicide-Tolerant Crops since 1996.

Oplinger and Minor think this potential yield difference is fully realized by scientists and most progressive growers with yield monitors, especially if they have side-by-side comparisons.

"In the industry, breeders know that they don't have yield parity with Roundup Ready varieties yet," Minor declares.

The most heard explanation for the yield lag is that the RR gene often has not been put into the most elite lines that some companies offer. A more plausible explanation, say some soybean breeders, is that in the rush to get RR lines on the market, many companies have not made enough backcrosses to capture all of the yield potential in the parent lines.

"The breeders have been caught with trying to turn things out as quickly as reasonably possible to stay competitive," says Oplinger.

In either case, the conventional wisdom says that will be corrected in a reasonably short time, and yield lags will be eliminated.

"I don't see any reason not to have yield parity in the future with this technology," says Minor.

Oplinger and Minor don't think average soybean growers realize or believe that there is a yield lag, on average, for RR varieties. Or if the growers do, they do not care because of the ease and effectiveness of the weed control they've gotten.

Oplinger points out that about a million acres of RR beans were grown in 1996. That increased to 9 million in '97 and over 25 million acres in '98.

"Therefore, if performance is to be measured by grower acceptance, then RR soybean varieties have certainly performed well," says Oplinger. "Also, the use of these varieties has solved some major weed problems for some growers."

Veteran weed scientists can't recall any herbicide product launch that created the excitement and acreage explosion that RR soybeans created. And grower surveys by Monsanto continue to show extremely high grower satisfaction. Postharvest surveys of RR soybean growers showed that nearly nine of 10 plan to grow some RR soybeans in 1999. With hundreds of new RR varieties available, Monsanto officials expect RR acreage to increase again this year.

In university variety performance trials, weed control has to be virtually perfect in order to measure true genetic yield potential of varieties, Minor points out. For average growers, weed control on larger acreages is not always that good, especially if weather problems occur.

"A farmer may lose the potential yield advantage of good conventional varieties if he doesn't have real good weed control. And if he has weeds he has not been able to effectively control with conventional herbicide programs, then he should give the Roundup Ready system serious consideration. That's because he is likely to lose more due to poor weed control than to the possible yield lag with the Roundup Ready system."

Oplinger urges growers to take a pencil to all the factors, including the yield lag, herbicide costs, technology fee, etc. Then figure out any bottom-line differences for their operations.

So, what's the take-home message of all of this?

If you are going to grow RR soybeans, study the variety performance tests from your state university, private companies or farms to try to pick the highest-yielding RR varieties with the defensive packages needed for your farm. If you don't, you could be sacrificing maximum

yield for ease of weed control.

"We have identified some Roundup Ready varieties, both from a yield and defensive package stand point, that growers definitely ought to consider," Oplinger concludes. "There is information out there to pick those varieties that best fit your needs - and your bottom line."

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Back to Top